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NEPHROLOGY FORUM

The initiation and progression of sickle cell nephropathy

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CASE PRESENTATION

A 35-year-old African-American man with sickle cell disease (SCD) and end-stage renal disease (ESRD) began chronic hemodialysis 8 years ago. He averages one to three pain crises per year and requires an average of two units of packed red blood cells per month to maintain a hemoglobin of 7 to 8 g/dL despite receiving 200 U/kg of subcutaneous recombinant erythropoietin per dialysis treatment and having no evidence of external blood loss. He had one episode of acute chest pain syndrome that resolved with conservative management. His hemoglobin electrophoresis reveals 4% to 6% hemoglobin F with >90% hemoglobin S. He refused treatment with hydroxyurea because of fear of side effects. At age 14, he had had microscopic hematuria and dipstick-positive proteinuria; his plasma creatinine concentration of 0.7 mg/dL was normal for his age and body mass. At age 19, his plasma creatinine was 1.4 mg/dL, and it progressively increased thereafter until he began hemodialysis at age 28. He has not had a renal biopsy.

The patient had five brothers and three sisters. Two sisters had SS hemoglobin genotype, one brother and one sister had SC, two brothers had AC, and two brothers had AS. One of his two SS sisters began hemodialysis last month at age 22, and the other SS sister died at age 40 for reasons unknown to the patient; she never required renal replacement therapy. His SC brother died at age 60 while on hemodialysis. The remaining SC sister is 50 years old and does not have renal failure, according to the patient. The AC and AS brothers are 41 to 52 years old and all are healthy without renal failure, according to the patient.

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Key words: papillary necrosis, focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, end-stage renal disease.

DISCUSSION

DR. DONALD E. WESSON (*Chief, Combined Program in Nephrology and Renal Physiology; and Chairman, Department of Internal Medicine, Texas Tech University Health Sciences Center, Lubbock, Texas, USA*): Patients with sickle cell disease (SCD) are living longer [1], but longevity of African-Americans with SCD remains considerably shorter than that in blacks without SCD [2]. African-American men and women with SCD examined between 1978 and 1988 lived an average of 42 and 48 years, respectively [2], compared to 65 and 74 years for black men and women in the general US population during a similar time period [3]. Renal failure occurs in 4% to 18% of SCD patients [4, 5] but in only <1% of the US population [6]. The average age at onset of ESRD in SCD patients from 1992 to 1997 was 40 years compared to 64 years for patients without SCD (Abbott KC, Hyolito I, Agodoa LY, personal communication). Thus, SCD patients develop ESRD more frequently and at an earlier age compared with the non-SCD US population. Renal failure is an important determinant of early death in SCD [2], so reducing its frequency might increase the longevity of these patients. Alternatively, improvements in SCD management that increase longevity but do not reduce ESRD risk potentially increase ESRD prevalence. As with other forms of ESRD, prevention is the goal, but designing effective preventive strategies requires an improved understanding of the mediating factors for initiation and progression of SCD-associated renal disease.

In this review, I will explore factors that might initiate SCD nephropathy and mediate its progression toward ESRD. I will offer a hypothesis for initiation and progression of SCD-associated nephropathy that can, and hopefully will, be tested. Because progression to ESRD in SCD-associated renal failure has features common to those of other causes of renal failure, a better understanding of renal disease progression in SCD-associated

renal failure might shed light on general mechanisms that can be applied to other diseases. I will focus this discussion on homozygous (SS) SCD, not on the many variants of this disease in which SS hemoglobin is combined with other hemoglobin subtypes.

The major pathologies in SCD-associated renal failure are papillary necrosis (PN), focal segmental glomerulosclerosis (FSGS), and type 1 membranoproliferative glomerulonephritis (MPGN) [7]. A frequent complication of SCD, PN was documented in 15% to 40% of patients with SCD undergoing urography to work up symptoms or urinary abnormalities, usually gross hematuria [8, 9]. Moreover, the frequency of PN among asymptomatic SCD patients who systematically underwent urography was 67% [10]. When PN is detected incidentally in SCD, it seldom is associated with a measurable decline in GFR [10], but we will look at how PN might set the stage for subsequent renal failure in SCD. By contrast, renal biopsies in patients [5, 7, 11, 12] and autopsy studies [13] suggest that FSGS is the most common cause of renal failure in SCD. Moreover, clinicopathologic studies show that the early lesion of most forms of SCD nephropathy is glomerular enlargement with progressive development of perihilar FSGS [7, 11]. These data support FSGS as the major cause of SCD nephropathy (Fig. 1). Although MPGN in SCD is morphologically similar to the classic variety of this disease, few cases have the characteristic immune deposits, particularly in the early stages [7, 11]. The etiology of MPGN in SCD is poorly understood, but it is not the major cause of renal failure in SCD [7]. Consequently, the focus of this discussion will be on FSGS as the major cause of SCD nephropathy and on PN as the possible prodrome for the subsequent appearance of FSGS.

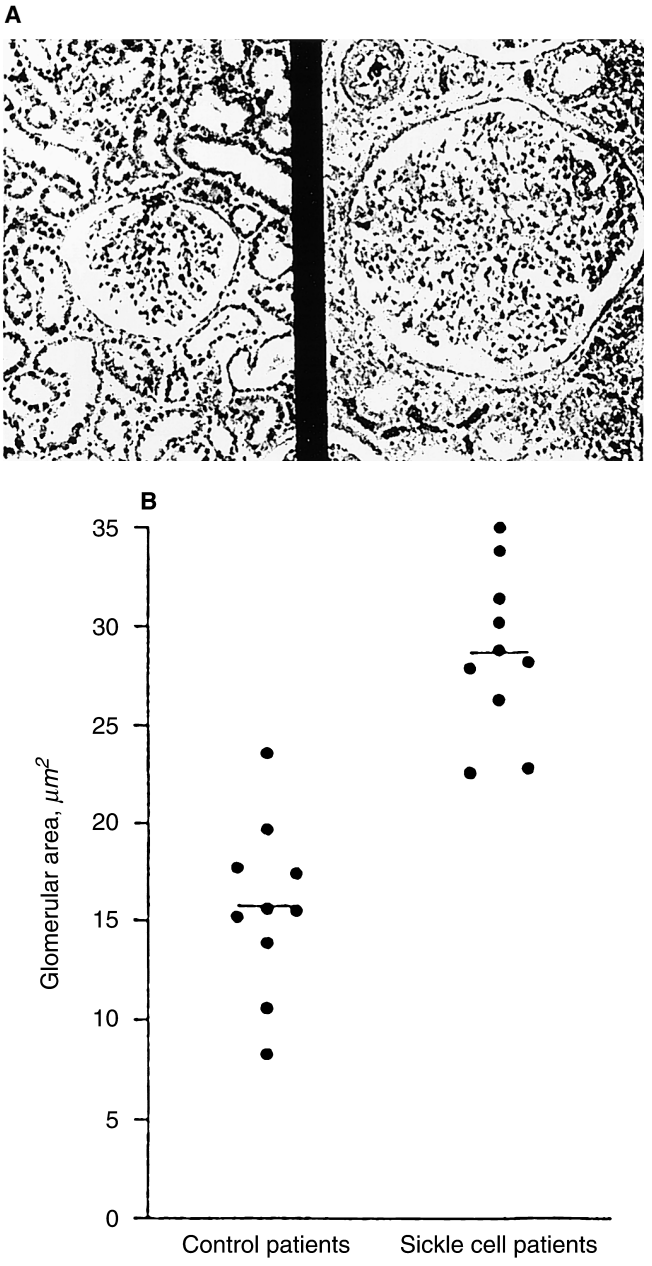

Fig. 1. (*A*) Glomerulus from a patient with sickle cell disease (right) is much larger than one from an age-matched control (left) at the same magnification. (*B*) Glomerular area indicated on the ordinate is significantly larger in sickle cell patients than control. (From Ref. 13.)

SCD features that might contribute to FSGS

The mechanisms by which FSGS specifically and glomerulosclerosis in general develop are incompletely elucidated, but many factors associated with FSGS are common in SCD. Three such characteristics might individually or in combination lead to FSGS and subsequent SCD-associated renal failure. *First*, recent data suggest that SCD is a state of oxidative stress [14]. In an animal model of SCD, initiation, progression, and resolution of the vaso-occlusive episodes that characterize SCD comprise features of ischemia-reperfusion injury with the associated chronic inflammatory response that includes activation of white blood cells and a local increase in the oxidative state [14]. Activated monocytes/macrophages of bone marrow origin play an important role in the pathogenesis of glomerulosclerosis in experimental animals [15] and in humans [16]. In addition, oxidized plasma lipoproteins are implicated in the pathogenesis of glomerulosclerosis both in experimental animals [17] and humans [18]. *Second*, children and young adults with SCD commonly have supranormal levels of glomerular filtration rate (GFR), that is, hyperfiltration [19, 20]. This phenomenon is associated with the subsequent development of FSGS in human and animal models of this disease [21]. Indeed, the GFR of younger patients with SCD is higher than that of age, weight, and gender-matched individuals without SCD [19] but the GFR subsequently falls to the normal range for most middle-aged SCD patients [20]. In addition, as I mentioned earlier, glomerular enlargement, a pathologic correlate of hyperfiltra-

tion, is a common early lesion of SCD nephropathy [11]. *Third*, 20% to 30% of patients with SCD have proteinuria [7], and chronic exposure of renal tubular epithelium to high levels of filtered plasma proteins might cause tissue injury with a subsequent inflammatory response [22]. Remuzzi and Bertani suggest that filtered plasma proteins taken up by tubular epithelium stimulate inflammatory genes and release inflammatory and vasoactive substances into the renal interstitium that induce scarring and sclerosis [22]. These three features of SCD likely contribute synergistically to the high rate of renal failure in these patients. Also, that only a minority of SCD patients develop renal failure suggests that genetically determined activity of non-SCD genes acts with these three factors to determine the risk of renal failure in an individual with SCD.

Papillary necrosis

Papillary necrosis in SCD can present dramatically with renal colic, gross hematuria, and acute renal failure with or without urinary tract obstruction [7, 8, 23]. More commonly, PN in SCD follows a more protracted course of months to years interrupted by recurring episodes of gross hematuria and/or urinary tract infection [7, 23]. Papillary necrosis in SCD is commonly asymptomatic, and its frequency among asymptomatic SCD patients is probably high [10]. Patients with SCD who develop symptomatic PN sometimes have blood clots and/or sloughed papillary tissue in their urine, but this finding was infrequent in one large series, and acute urinary tract obstruction was even less frequent [23]. Altogether, PN is commonly diagnosed as an incidental finding in the urologic workup of patients with SCD and is seldom a cause of renal failure at the time it occurs. Nevertheless, PN might set the stage for the subsequent development of FSGS, as I will discuss later.

Let's turn our attention to the pathogenesis of PN in SCD. Papillary necrosis in SCD is mediated by blood vessel occlusion that causes infarction and necrosis of papillary tissue, the degree of which varies [7]. In most patients with SCD, the infarcted areas are small, and renal function, as measured clinically, usually is not affected [23], at least not immediately. Less commonly, SCD patients have more widespread necrosis of the papilla [24] due to more extensive infarction. Given that these patients all have the same SS genotype, these data suggest that individual variability in non-SCD genes helps determine the degree of blood vessel occlusion with infarction. Thus, while sickle hemoglobin is caused by a single mutation, SCD could be a multi-gene disease [25], and this concept helps account for the variable severity of disease, including the degree of PN, among SCD patients. I will return to this concept later.

Both SS hemoglobin polymerization and red blood cell (RBC) vascular adhesion mediate blood vessel occlusion in SCD [26]. Vessel occlusion is also affected by local production of cytokines that alter local factors in a way that augments vascular occlusion [26]. Examples of these local factors in animal models of SCD include recruitment of pro-inflammatory cells [14] and local release of vasoconstricting agents like endothelin [27], each phenomenon potentially impeding escape of sickled RBCs. Individual variation in SS hemoglobin polymerization, RBC vascular adhesion, and cytokine production might determine the degree of PN in patients with SCD.

Hemoglobin SS crystallizes to form the sickled RBCs by a process with the characteristics of nucleation-initiated polymerization [28]. The most important step in the cascade leading to SS hemoglobin polymerization is formation of a critical nucleus of tetramers, after which polymerization proceeds rapidly. The time required to form this critical nucleus, known as the "delay time," is ordinarily greater than the transit time through the microcirculation of most tissues, thereby sparing SCD patients from ongoing massive and constant RBC sickling. The risk for SS hemoglobin polymerization is increased by low O_2 tension, hypertonicity, and low pH [28], all characteristics of the renal medulla [7]. Nevertheless, individual variability in the degree of PN in patients with the same SCD genotype suggests that additional factors are important. For example, genetically controlled activity of RBC membrane cation transporters [26] determines RBC volume and thereby intracellular hemoglobin concentration [29], an important determinant of the sickling risk. Also, SCD patients with higher levels of fetal hemoglobin or of hemoglobin A2 have a lower risk for SS hemoglobin polymerization [30]. The level of synthesis of these hemoglobin subtypes is under individual genetic control separate from that of SS hemoglobin [30, 31]. Individual variation in the expression of these and probably other factors in SCD patients helps determine the degree of SS hemoglobin polymerization and thereby infarction of papillary tissue with subsequent PN.

In addition to hemoglobin S polymerization, the degree to which sickled RBCs adhere to vascular endothelium is an important component of the cascade leading to blood vessel occlusion in SCD [26]. Sickled RBCs adhere to cultured non-human vascular endothelial cells [32], to cultured human vascular endothelial cells [33], and to vascular endothelium of in vivo animal models of SCD [34]. Patients with SCD have elevated plasma levels of the endothelial adhesion proteins von Willebrand factor (vWF), vascular endothelial adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) [35, 36], and vWF and VCAM-1 mediate adhesion of sickled RBCs to vascular endothelium [26]. Patients with SCD also have plasma markers of vascular endothelial injury [36], and endothelial injury itself increases vWF and VCAM-1 release from the vascular endothelium [36, 37]. In addition, sickled RBCs induce

expression of VCAM-1 and ICAM-1 in human vascular endothelial cells in vitro [38]. Monoclonal antibodies that block adhesion molecules inhibit adhesion of sickled RBCs to vascular endothelium [39]. This finding further demonstrates the importance of these molecules in mediating adherence of sickled RBCs to the vascular endothelium. Adhesion molecules also mediate polymorphonuclear leukocyte attachment to vascular endothelium, and attachment of these larger and less deformable cells dramatically impedes RBC passage, increasing the risk for sickling and vascular occlusion [40]. Furthermore, immature, larger RBCs are more likely than mature ones to adhere to vascular endothelium [26]. There is individual variability among SCD patients in their marrow response to the increased RBC destruction that characterizes SCD [31]; variability in the release of immature RBCs thus might contribute to the individual variability in the degree of vaso-occlusive disease, including PN, among SCD patients. Additionally, the degree of expression of endothelial cell integrins appears to be genetically determined, and adhesion of RBCs and WBCs to the vascular endothelium is determined by the degree of expression of these substances [26].

Although PN in SCD is not commonly associated with reduced renal function, some associated features might "set the stage" for the subsequent development of FSGS. Supporting this hypothesis is the fact that FSGS occurs as a late consequence of many disease processes in humans that cause PN [41] and in the bromoethylamine (BEA)-induced experimental model of PN [42]. Two characteristics of PN that might facilitate the subsequent development of FSGS include glomerular hyperfiltration and increased filtration of plasma proteins. Because juxtamedullary nephron damage is a common pathologic feature in young SCD patients [43], and GFR in these young patients generally is not reduced [19], surviving cortical nephrons in young patients with SCD and variable degrees of PN might have a higher-than-normal GFR. Indeed, patients with SCD have higher GFRs than do age-matched controls [44]. In addition, single-nephron GFR in the surviving cortical nephrons is higher than control in the BEA model of PN, in which papillary and deep nephron function is severely damaged [45]. Higher GFR in SCD is associated with increased glomerular permselectivity and increased glomerular ultrafiltration coefficient [44]. On the other hand, urinary protein excretion in SCD inversely correlates with GFR, and proteinuria is associated with a reduced glomerular ultrafiltration coefficient [46]. Furthermore, SCD patients with a more severe decrease in GFR have an increased glomerular permeability to proteins of all sizes as well as a markedly reduced glomerular ultrafiltration coefficient [47]. Non-nephrotic proteinuria is common in humans with PN of various causes [41] and is present in experimental animals with the BEA-induced model of PN [42]. The patient presented today had macroproteinuria many years before his plasma creatinine concentration was noted to exceed the normal range. The studies I have mentioned are consistent with a scenario in which hyperfiltration combined with increased glomerular filtration of plasma proteins contributes to FSGS and a progressive decline in GFR.

Focal segmental glomerulosclerosis

Focal segmental glomerulosclerosis, associated with many pathologic processes including SCD, is the final common pathway toward ESRD in many renal parenchymal diseases [41]. The lesion has been divided into "classic" and "collapsing" varieties according to its pathologic appearance. The two varieties are thought to be due to different mechanisms. Classic FSGS begins with segmental "tuft adhesions" of the glomerular capillary to the surrounding parietal epithelium that progress to segmental collapse of the glomerular capillary with eventual sclerosis that can subsequently involve the entire glomerulus [48]. This is the most common variety of FSGS and includes that described in SCD. By contrast, the collapsing variety begins with global collapse of the capillary tuft and leads to global sclerosis [48]; it is best exemplified by HIV nephropathy. Although the pathogenesis of FSGS is incompletely understood, the classic variety of this disorder is characterized by: (*1*) glomerular hypertrophy and/or hyperplasia; (*2*) glomerular capillary hypertension with or without systemic hypertension; (*3*) podocyte damage; and (*4*) mesangial damage including "insufficiency" and mesangiolysis [48]. Two other features common to FSGS are variable degrees of mesangial cell proliferation and/or matrix expansion along with surrounding tubular atrophy and interstitial fibrosis [49]. Many SCD features directly or indirectly lead to all these events and thereby increase the risk of FSGS for patients with SCD.

Hyperfiltration with glomerular enlargement commonly precedes the onset of FSGS both in animal and human models of this disease [16, 41] including SCD [11, 50]. The cause of hyperfiltration in SCD is unknown but might be mediated by an increased production of vasodilating prostaglandins [51], prostacyclin [52], and/or nitric oxide [53]. Some experimental PN models in which FSGS subsequently develops are associated with increased glomerular capillary hydrostatic pressure [44]. This increased hydrostatic pressure might lead to FSGS by directly or indirectly damaging podocytes, mesangial cells, and endothelial cells of the glomerulus.

Glomerular podocyte damage is an important event in progression toward FSGS [48]. Unlike other glomerular cells, podocytes appear to be unable to replicate, so when they are damaged, they are irreversibly lost [48]. Severe podocyte damage leads to their detachment from the underlying glomerular basement membrane. Because their

interdigitating foot processes contribute importantly to maintaining selectivity of the glomerular filtration barrier, podocyte loss increases passage of plasma proteins into the urinary space [54]. In addition, podocytes along with mesangial cells and their matrix contribute to the structural integrity of the glomerular capillary wall, which sustains intra-luminal hydraulic pressure higher than that in typical capillary systems [55]. Without the external structural support provided by podocytes, the glomerular capillary wall balloons outward, and the adjacent basement membrane loses its folding characteristics [48]. The exposed, ballooning, and unfolded basement membrane attaches to the parietal epithelium of Bowman's capsule to form a tuft adhesion [48].

The segmental glomerular sclerosis that characterizes FSGS begins with formation of the tuft adhesion, after which the adjacent glomerular capillary wall hyalinizes; this process leads to a progressive decrease in its lumen diameter with eventual obliteration [48]. The basement membrane surrounding the glomerular capillary and the attached parietal epithelium eventually rupture. This tear permits extravasation of capillary luminal contents into a space created between the external side of the parietal epithelium and the basement membrane surrounding Bowman's capsule, as well as into the renal interstitium [48]. Experimental models of FSGS show that activated leukocytes including monocytes/macrophages enter this space [56], where they might induce sclerosis [57] and thereby promote the glomerulosclerosis that characterizes FSGS. This space eventually fills with matrix material contributed by mesangial cells and possibly by remaining podocytes [48]. The sclerotic periglomerular space is initially segmental but spreads circumferentially to involve the entire glomerulus and increasingly compresses the capillary tuft until the glomerulus becomes non-functional or nearly so. Mesangial damage with or without mesangial cell proliferation commonly accompanies FSGS [16, 49].

In addition to the glomerular changes, interstitial inflammation with sclerosis is a consistent feature of FSGS [48] and might be mediated by at least three mechanisms. *First*, the damaged glomerulus permits capillary contents to extravasate into the renal interstitium as described earlier, evoking an inflammatory reaction [48]. *Second*, cytokines released by invading activated leukocytes facilitate progression of interstitial inflammation to sclerosis and fibrosis [58]. *Third*, severely damaged glomeruli with patent capillary loops have dramatically increased filtration of plasma proteins [55] that might evoke an inflammatory response from renal tubular epithelial cells [22]. The interstitial inflammatory response caused by these factors induces adjacent interstitial cells to proliferate and form a continuous layer of thin sheet-like fibroblasts that separate the sclerotic glomerulus from the interstitium [48].

Although many components of the cascade of events

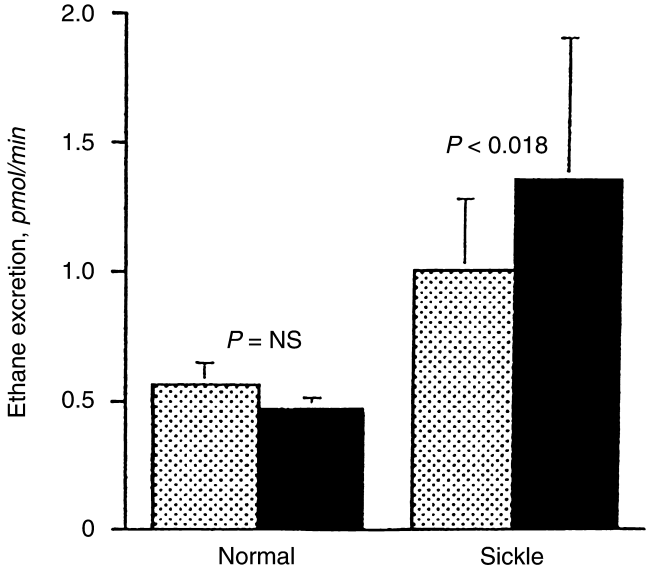

Fig. 2. Compared to normal, the transgenic mouse model of sickle cell disease produces higher levels of ethane, a measure of the production of reactive oxygen species. This increase is most evident after the animals are reoxygenated (■) following hypoxia; (▩) the baseline period. (From Ref. 67.)

that lead to FSGS are known, less clear are the events that initiate this cascade. Recent studies support injury to the glomerular capillary endothelial cell as a critical first step on the road to FSGS [59, 60]. Patients with SCD suffer varying degrees of ongoing vascular endothelial injury. They have elevated plasma levels of markers that reflect vascular endothelial injury [36] and elevated levels of denuded and circulating vascular endothelial cells during crises [61] and even when asymptomatic [36]. Patients with SCD also have histologic damage to their vascular endothelium [62]. Injury to the glomerular endothelium might be caused by high shear stress associated with hyperfiltration [21]. It is important to note that animal models of SCD have increased production of reactive oxygen species (ROS) (Fig. 2) that cause vascular endothelial damage [14]. Following vaso-occlusive injury, this same model (Fig. 3) has increased emigration of activated leukocytes that produce ROS when they bind to vascular endothelial cells expressing cell adhesion molecules induced by exposure to sickle RBCs in vitro [38] and in vivo [14]. These recruited leukocytes produce ROS that directly damage vascular endothelium [14]. Sickle RBCs also produce ROS that damage vascular endothelium [63, 64]. These SS RBCs adhere to vascular endothelium [34] and do so more readily than do RBCs with normal hemoglobin [33]. Hemoglobin SS loses heme faster than normal hemoglobin due to accelerated auto-oxidation [63]. Accelerated auto-oxidation of SS hemoglobin leads to increased production of ROS [64] and to increased deposition of iron that catalyzes ROS

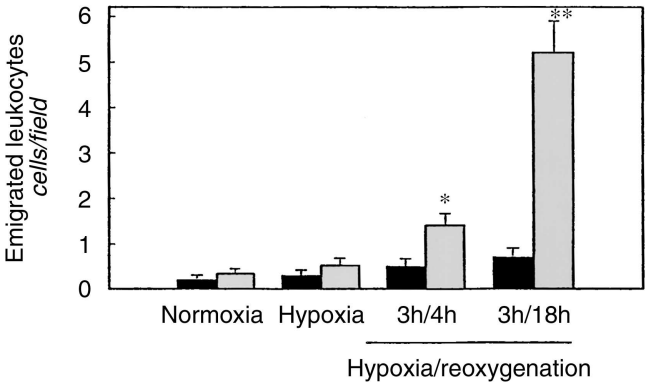

Fig. 3. Emigration of leukocytes is greater following hypoxia/reoxygenation in the transgenic mouse model of sickle cell disease (□) than in controls (■). (From Ref. 14.)

Table 1. Factors that likely cause FSGS in SCD

Increased production of reactive oxygen species due to
Hypoxia/reoxygenation
Recruitment of activated leukocytes
Auto-oxidation of sickle hemoglobin
Hyperfiltration of cortical nephrons due to
Papillary necrosis with medullary nephron damage
Cytokines from damaged vascular endothelium and activated leukocytes
Chronic exposure of renal tubular epithelium to high levels of filtered plasma proteins due to
Cortical nephron hyperfiltration
Podocyte damage
Glomerular tuft adhesions with glomerulosclerosis

Abbreviations are: FSGS, focal segmental glomerulosclerosis; SCD, sickle cell disease.

production in the membrane of SS RBCs [65]. The oxidative damage to vascular endothelium induced by SS RBCs also induces expression of cell surface adhesion molecules, diapedesis of monocytes, and adherence of additional SS RBCs to the vascular endothelium [66]. All these events occur in the transgenic SCD model [14, 67] and all are ameliorated by inhibitors of ROS production [67].

Investigators have suggested analogies between glomerulosclerosis and atherosclerosis [68], the latter being another pathogenetic process in which vascular endothelial injury is an important initial step [69] and for which oxidative stress augments progression [70]. Activated macrophages with foam cell formation contribute to progression of glomerulosclerosis and atherosclerosis [68]. Oxidized low-density lipoproteins found in atherosclerotic plaques [70] and in sclerotic segments of human glomeruli with FSGS [18] stimulate production of extracellular matrix by vascular smooth muscle cells in atherosclerotic plaques [70] and similarly stimulate matrix production by glomerular mesangial cells [71]. Oxidative stress injures glomerular mesangial cells [72] and podocytes [48] and leads to sclerotic responses in both cell types [48, 72] as well as injures vascular endothelium, as I discussed earlier. Evidence supports the importance of oxidative stress to renal injury in a transgenic mouse model of SCD; inhibition of nitric oxide (NO) synthesis and NO-mediated oxidant stress ameliorated renal cellular injury [73]. These data suggest that preventive strategies for atherosclerosis are effective in preventing initiation and progression of FSGS in patients with SCD.

What causes FSGS in SCD? Hypothesized mechanisms for SCD nephropathy must include factors that (*1*) initiate the cascade toward FSGS and facilitate its progression to ESRD, and (*2*) explain why only a minority of SCD patients develop renal failure. Table 1 outlines possible factors that contribute to FSGS in SCD. Chronic vascular endothelial injury induced by ROS might be a key factor in both the initiation and progression of SCD nephropathy due to FSGS. This injury causes adhesion of sickled RBCs to vascular endothelium and increases vaso-occlusive disease. The renal medullary environment (hypoxic, hypertonic, and acidic) favors polymerization of SS hemoglobin and greater microvascular occlusion in the papilla compared to other tissues, causing PN and medullary nephron damage. The resulting hyperfiltration of cortical nephrons, as well as ROS, injure glomerular cells and initiate the sclerotic process, the progress of which is promoted by cytokines from activated WBCs and possibly from injured vascular endothelium. Increased filtration of plasma proteins due to glomerular damage induces interstitial inflammation that is facilitated by these same cytokines. Glomerulosclerosis combined with interstitial inflammation complete the picture of FSGS. Renal failure is hypothesized to occur in only a minority of SCD patients because of genetically determined non-SCD genes that mediate the degree of vaso-occlusive disease, which in turn helps determine the level of ROS. Examples of these genetically determined factors include (*1*) the fetal hemoglobin level; (*2*) marrow production of immature RBCs that are more adherent to vascular endothelium; (*3*) adhesion molecule expression in response to vascular endothelial injury; and (*4*) activity of RBC cation transporters, which helps determine cell SS hemoglobin concentration. One of the two SS siblings of today's patient developed ESRD at age 22; the other died at age 40 of unknown causes but without ESRD. Although anecdotal, these data support the contribution of non-SCD genes to the development of SCD nephropathy.

Preventing SCD nephropathy

Investigations that have affirmed at least some elements of the hypothesis offered to account for the mechanisms mediating SCD nephropathy have suggested potential preventive strategies. Interventions that might ameliorate hyperfiltration in SCD and thereby reduce

the risk of nephropathy include inhibitors of prostaglandin and/or prostacyclin synthesis and inhibition of NO synthase. The latter intervention ameliorated renal cellular injury in an experimental model of SCD [73]. On the other hand, prostaglandin inhibitors should be used with caution in patients with SCD given their nephrotoxic potential and demonstrated antinatriuretic effect of these agents in SCD [51]. In the renal ablation animal model of FSGS characterized by glomerular hyperfiltration and hypertension, angiotensin converting enzyme (ACE) inhibitors decreased glomerular hyperfiltration and hypertension and reduced the risk for subsequent development of FSGS [74]. Given the ability of ACE inhibitors to retard progression of renal diseases mediated by glomerulosclerosis [75], investigators are currently testing the efficacy of these drugs to reduce the likelihood of renal failure in patients with SCD [7]. Interventions that reduce oxidant stress mediated by ROS also might reduce the risk for nephropathy in SCD. Anti-oxidant therapy reduces cardiovascular end points in patients with ESRD [76]. Given the similarities between FSGS and atherosclerosis that I described earlier, anti-oxidant therapy might retard FSGS progression [69]. Finally, decreasing the traffic of plasma proteins across the glomerular basement membrane in patients with SCD might reduce the risk of nephropathy. It is encouraging that short-term ACE inhibition therapy reduces urinary albumin excretion in SCD patients [7, 77]. Longer-term studies currently underway should determine whether this ACE inhibitor-induced reduction in urinary albumin excretion translates into a reduced risk of renal failure.

QUESTIONS AND ANSWERS

Dr. John T. Harrington (*Dean, Tufts University School of Medicine, Boston, Massachusetts*): Can one get FSGS in sickle cell disease if one has no papillary necrosis?

Dr. Wesson: Autopsy studies show that every patient with FSGS has at least some degree of papillary necrosis, and in most cases it is severe. There is a very good connection between FSGS and papillary necrosis in sickle cell disease.

Dr. Neil Kurtzman (*Texas Tech University Health Sciences Center, Lubbock, Texas*): Don, you have convinced me about the glomerular disease, but I would like to focus on some of the tubular abnormalities associated with SS hemoglobin. As you know, since you were there when it happened, we showed that the BEA model of papillary necrosis does not have hyperkalemic renal tubular acidosis [78]. De Fronzo showed that patients with hemoglobin S and normal renal function have a defect in potassium excretion [79]. We subsequently showed that patients with abnormal renal function and a variety of hemoglobin S pathologies have hyperkalemic distal tubular acidosis similar to that seen in urinary tract obstruction [80]. These data suggest that hemoglobin S itself might be toxic to renal tubular function. Given that the work to which I am alluding is more than 20 years old, is there anything new about the role of hemoglobin S in impairing renal function?

Dr. Wesson: Hemoglobin S is a powerful oxidant and contributes to oxidative stress in SCD [63, 64]. Extracellular hemoglobin S might exacerbate the interstitial damage in SCD that can lead to tubular injury. As I mentioned, one can also make a case for proteinuria-induced renal tubule injury.

Dr. Barry Sobel (*Texas Tech University Health Sciences Center, Odessa, Texas*): If proteinuria due to increased capillary pressure is the initiating factor in FSGS, why do patients with analgesic nephropathy develop end-stage renal disease without developing severe proteinuria?

Dr. Wesson: First, let me say that I believe that papillary necrosis is *an* initiator and not *the* initiator of FSGS. Let me follow by stating my bias that proteinuria is important, if not critical, in the development of FSGS in sickle cell nephropathy. Second, patients with analgesic nephropathy typically have low-grade proteinuria, less than 2 g/day, even in early stages of this process [39]. Urinary protein excretion reflects that amount which escapes tubular reabsorption so that the renal tubules have been exposed to levels that are much higher than those in the urine.

Dr. Sharma Prabhakar (*Texas Tech University Health Sciences Center, Lubbock*): Dr. Wesson, you mentioned that hyperfiltration is an important factor in the development and progression of sickle cell nephropathy. You also alluded to the role of different factors including nitric oxide in explaining the hyperfiltration. Norman Bank's group has shown activation of inducible nitric oxide synthase (iNOS) in transgenic models [53], so increased nitric oxide production might explain the hyperfiltration. On the other hand, the same group subsequently showed that the activation of iNOS in the same model under hypoxic circumstances leads to formation of peroxynitrate and superoxide, which, in turn, could cause apoptosis and the structural damage that characterizes sickle cell nephropathy [73]. Traditionally, iNOS-induced formation of peroxynitrate and superoxide leads to decreased nitric oxide levels. In addition, several investigators have shown that the acidosis that accompanies hypoxia also inhibits iNOS [81]; thus, the net effect of hypoxia on iNOS is unclear. Consequently, are there mediators of hyperfiltration in SCD that are more important than nitric oxide? In particular, what is the role of the renin-angiotensin system in sickle cell nephropathy?

Dr. Wesson: As I alluded to, at least two other systems contribute to hyperfiltration in sickle cell disease. One is the prostaglandin system. Allon et al showed that prostaglandin inhibitors decrease hyperfiltration in SS pa-

tients and suggested a role for prostaglandins [51]. Additionally, that prostacyclin levels are increased in SCD [52] suggests a role for this vasodilator with NO-mediated actions. The only study of which I am aware that examines the GFR response of SCD patients given angiotensin-converting-enzyme inhibitors showed no GFR change in response to this intervention [5]. These data suggest that the renin-angiotensin system does not play a major role in the hyperfiltration of SCD.

Dr. Melvin Laski (*Texas Tech University Health Sciences Center, Lubbock*): I'd first like to comment relative to the prior question about analgesic nephropathy. Analgesic nephropathy occurs in later adulthood rather than in childhood. Consequently, the difference between development of FSGS in SCD and analgesic nephropathy might be similar to the difference between being born with one kidney and developing FSGS compared to losing a kidney in adulthood and not developing FSGS. The difference might be a matter of the age at which the renal mass was lost. My question goes to the clinical correlates in nephropathy and SCD. Is there any relationship between the clinical course of SCD and the risk for developing nephropathy? Specifically, is there any correlation with hemolytic crisis and exposure to acellular hemoglobin?

Dr. Wesson: The only study of which I am aware that addresses the relationship of the number of pain crises and the risk for subsequent sickle cell nephropathy is by Powars et al [4]. In this study, patients with an increased number of pain crises did not have an increased risk of renal failure.

Dr. Everardo Cobos (*Texas Tech University Health Sciences Center, Lubbock*): Is there any evidence that hydroxyurea can ameliorate or prevent the progression of the renal failure?

Dr. Wesson: The answer is not known. The suspicion is that it can, but no one has published a large enough series of patients followed long enough to be able to answer the question. If vaso-occlusive disease is important in the development of SS nephropathy, then the increased hemoglobin F induced by hydroxyurea should reduce the risk for SS nephropathy. This suggests that the drug would be most useful in preventing SS nephropathy if given in early childhood of SS patients.

Dr. Juan Sarria (*Texas Tech University Health Sciences Center, Lubbock*): What should the primary care physician do with the SCD patient who develops hematuria or proteinuria but is normotensive?

Dr. Wesson: That is a question for which we have insufficient data to adequately answer. Given that ACE inhibitors reduce proteinuria in SCD [74], I suggest giving such a patient ACE inhibitors. Other data suggest that oxidant stress is an important contributor, and some would suggest that anti-oxidants such as vitamin E should be given. Nevertheless, I am unaware of data showing that vitamin E reduces oxidant stress in SCD. Therefore, a reasonable and relatively benign intervention for the patient described would be ACE inhibitors.

Dr. Erdal Diri (*Texas Tech University Health Sciences Center, Lubbock*): You addressed the potential role of VCAM-1 overexpression in the pathogenesis of SCD-related renal disease. What is the role of immunosuppressant therapy in SCD, especially that targeting these molecules?

Dr. Wesson: Reducing the response of the vascular endothelium to produce adhesion molecules with immunosuppression therapy sounds like a good idea, but I am aware of no data regarding this intervention. In the absence of such data I am hesitant to recommend an intervention that is less benign than ACE inhibitors and vitamin E.

Dr. Harrington: One of the things you commented on in the case presentation was this patient's need for erythropoietin and frequent blood transfusions. Do we know whether the site of erythropoietin production within the kidney is particularly damaged in patients with sickle cell nephropathy?

Dr. Wesson: The only study of which I am aware that tangentially addressed this issue showed that erythropoietin production decreased in SS patients who developed renal failure [82].

Dr. Harrington: I believe that there is an increased incidence of uroepithelial carcinoma in patients with sickle cell disease. Does that happen in patients with other types of papillary necrosis?

Dr. Wesson: There is an increased incidence of uroepithelial cancer in patients with papillary necrosis, particularly that due to analgesic nephropathy [83].

Dr. Harmohan Singh (*Texas Tech University Health Sciences Center, Lubbock*): Dr. Wesson, you stated that FSGS is the predominant lesion mediating sickle cell nephropathy, but that a small minority of SS patients has membranoproliferative GN. In sickle cell nephropathy, does membranoproliferative GN progress faster to ESRD than FSGS does?

Dr. Wesson: There are no good prospective data to answer this question. The data that exist suggest that FSGS progresses faster to ESRD than does MPGN in sickle cell nephropathy.

Dr. Prabhakar: You cited studies showing that ACE inhibitors reduce proteinuria in SS patients and suggested that these drugs might slow disease progression. Are there any ongoing long-term studies with ACE inhibitors in sickle cell nephropathy? Second, has anyone looked at microalbuminuria as a predictor of progressive renal disease in SCD?

Dr. Wesson: Yes, Dr. Falk's group in North Carolina is investigating the effect of ACE inhibitors on the progression of SS nephropathy [7]. Powars showed that dipstick-positive proteinuria was predictive of nephropathy

[4], but I am not aware of studies that have examined the power of microalbuminuria to predict subsequent sickle cell nephropathy.

Dr. Laski: This is a question about current SCD management. I am well aware of this patient's course and the frequent need for transfusion. One of the consequences of that over many years is iron overload that is particularly difficult to manage. What are we to do when blood transfusions that maintain adequate blood counts also yield hemochromatosis?

Dr. Wesson: The case presented is my personal patient and I am currently facing that dilemma. We have unsuccessfully tried to convince him to take hydroxyurea. This can increase hemoglobin F and thereby reduce the vaso-occlusive disease that might be contributing to hemolysis and his ongoing need for blood transfusions. I am not aware of any other reasonable options.

Dr. Harrington: Are there are new ways of raising the hemoglobin F level besides hydroxyurea?

Dr. Wesson: This nephrologist is not aware of any ways to do that. However, more exciting are potential gene therapy approaches. Pawliuk et al just reported in *Science* that an HIV vector, re-engineered to deliver the hemoglobin A gene, successfully cured test mice over a 10-month period [84].

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